Evaluation of the interaction of vanadium with glutathione in human blood components

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Abstract: Metallo-elements including Vanadium (V) have strong affinity for sulfhydryl (-SH) groups in biological molecules including Glutathione (GSH) in tissues. Because of this fact it was of interest to further investigate the interaction of Ammonium Vanadate [NH_4VO_3] with Glutathione as a biomarker of toxicity and the role of Glutathione in the detoxification and conjugation processes in whole blood components including plasma and cytosolic fraction. Effects of different concentrations of Ammonium Vanadate [NH_4VO_3] on the level of reduced Glutathione in whole blood components (Plasma and Cytosolic fraction) were examined. GSH depletion in plasma and cytosolic fraction was Ammonium Vanadate's concentration-dependent. Depleted GSH level was more pronounced with more incubation time period. These findings show that changes in the GSH status produced by Ammonium Vanadate could be due to either by adduct formation of Vanadium and glutathione i.e. (V-SG) or by increased production of oxidized Glutathione (2GSH $+V^{+5} \rightarrow$ GSSG). This change in GSH metabolic status provides some information regarding the mechanism of toxicity by Ammonium Vanadate and the protective role of glutathione.

Keywords: Ammonium nanadate (NH_4VO_3), glutathione (GSH), Di,thiobis-dinitrobenzoic acid (DTNB), blood components, plasma, cytosolic fraction (CF), V^{+5} (penta-valant vanadium).

INTRODUCTION

Vanadium is an ultra trace metal, present in mammalian tissues at concentrations below 1μM (Nechay *et al.*, 1986). It has been suggested that in mammalian cell culture systems vanadium (V^{+5}) is biologically more active than vanadium (V^{+4}) in inducing toxic effects (O'neal *et al.*, 1979). Vanadium is known for its spasmogenic property in various smooth, cardiac and skeletal muscles. Potent vasoconstrictor effects of Vanadium *in vitro* as well as in animals were demonstrated in several laboratories (O'neal *et al.*, 1979). Vanadate has to enter the cell through the ionic pore and produce its effects on the contractile protein (Murphy, 1994). It has been found that vanadium-containing compounds exert potent toxic and carcinogenic effects, such as DNA damage and cell transformation (Huang *et al*., 1998; Cohen *et al*., 1992 and Sheu *et al*., 1992) In animal studies, vanadium compounds or vanadium containing air pollution particles have been shown to induce inflammation in the respiratory tract (Pierce *et al*., 1996; Carter *et al*., 1997). It has been reported that vanadium associated with air pollution particles, such as residual oil fly ash, can induce the synthesis and expression of inflammatory cytokines, such as interleukin-1 (IL-6), interleukin-8 (IL-8) and TNF-a ((Pierce *et al*., 1996; Carter *et al*., 1997; Dye *et al*., 1999 and Dong *et al*., 1998).

Glutathione a tripeptide is found in all forms of life and plays a protective role in the health of organisms, (Hermes-Lima *et al*., 1991). The functions of glutathione in the organism are associated with the thiol group (-SH). Glutathione acts as a substrate or co-substrate with a number of enzymes which exhibit Antioxidant properties in mammals, e.g. with glutathione S-transferase or glutathione peroxidase. At the same time it can react with $1O_2$, $HO^{\bullet}$ or $O_2^{\bullet}$ itself (Sies, 1993.). GSH takes part in the detoxification of xenobiotics and heavy metals (copper, silver, zinc vanadium), and forms complexes with them. Glutathione reactions with the radicals of organic substances (including free radicals of other molecules) may lead to their "repair", but also result in free glutathione radical formation (Bartosz, 1993). Glutathione can affect the oxidation-reduction condition of certain proteins through the non-enzymatic exchange of SH/SS groups (Lenartowicz *et al*., 1993). It appears that glutathione combines both enzymatic and non-enzymatic protection of cellular structures against oxidation (Bartosz, 1993). It has been reported that the cell can protect itself against Vanadate toxicity by reduced Glutathione-mediated conversion of vanadium (V) to vanadium (IV) (Sabbioni *et al*., 1992).

The present study examines the effect of Vanadium and Ammonium Vanadate (NH_4VO_3) on GSH metabolic modulation in whole blood components including plasma and cytosolic fraction *in vitro* as a model of an *in vivo* reaction.

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MATERIALS AND METHODS

Materials

NaOH (sigma), NaCl (fluka), Ammonium Vanadate (NH_4VO_3) (sigma), L-glutathione (GSH), Potassium Di-hydrogen Phosphate (Sigma), Disodium-Edetate (Merk), Dithio-bis, di-nitrobenzoic Acid (DTNB) Chloroform (Sigma), HCl, Diposible rubber-gluves, Ethanol (Sigma), siliconised-glass test-tubes, Sterile syringes (Surge Pharmaceuticals) Eppondolfs-tubes (Pyrex Germany), All chemicals were used for research work without any further purification. UV-Spectrophotometers of Model 1601 (Shimadzu), Centrifuge (H-200 Kokusan, Ensink Company of Japan), pH-meter (Model NOV-210, NOV Scientific Company Ltd. Korea).

Methodology

AmmoniumVanadate (NH_4VO_3) (Mol. Weight 116.58) Stock solution (1mM) was prepared by dissolving 5.83mg of ammonium Vanadate in 50ml of distilled-water. 9mg of NaCl were dissolved in 100ml of distilled-water for the preparation of 0.9%Nacl solution. Standard Glutathione solution (1mM) was prepared by dissolving 30.74 mg of glutathione in 100-ml of 0.1N-HCl.Di-thiobis,Di-nitrobenzoic acid (DTNB) (1mM) was prepared by dissolving 39.6mg of DTNB in 100 ml of phosphate buffer solution. Phosphate buffer solution (0.2M) was prepared by mixing 42.2-ml NaOH (0.2M) in 50-ml of monobasic potassium phosphate solution (0.2M), making the final volume up to 200ml water. pH was adjusted [0]to 7.6 by using Ph-meter.

Preparation and isolation of blood components

Isolation of plasma

12ml fresh venous blood was collected from a human healthy volunteer and was treated with 0.5ml Na-EDTA (500µl) to prevent clotting, one ml blood was taken and mixed with 1-ml of Ammonium Vanadate (200-2000µM) solution in separate test tubes and incubated for 10 minutes, in each tube the final concentration of Ammonium Vanadate was from (100-1000µM).Each of these 2ml samples were then centrifuged at 10000-rpm for five minutes. From the supernatant fluid, 0.8ml plasma was removed with a Pasteur pipette, transferred to sample tubes and kept on ice until analyzed. The packed cells were further processed for Cytosolic fraction. 1ml of venous blood was mixed with 1ml of 0.9% NaCl solution and then centrifuged to get control sample of plasma.

Isolation of cytosolic fraction of blood

The packed cells were washed twice with (0.9%NaCl) solution and the blood cells were lysed at 4ºC, with an equal volume distilled water for 1 hour. After lysis at 4ºC, 0.8 ml of cold mixture of chloroform, ethanol (3:5V/V) at 0ºC was added to 2ml of lysed cells to precipitate the hemoglobin, followed by 0.3 ml of distilled water. The resulting mixture was also centrifuged as mentioned above and the supernatant yellowish fluid (cytosolic fraction) was collected. The control sample containing 1ml of venous blood and 1 ml of 0.9% NaCl solution was also centrifuged for isolation of cytosolic fraction.

Determination of inorganic biological parameters

1. Plasma glutathione (Extracellular).
2. Lysate glutathione (Intracellular).

All glutathione estimations were carried out using the modified standard Ellman's method (Ellman's 1959) as follows:

2.3 ml buffer was mixed with 0.2ml of sample (plasma/Cytosolic fraction of blood) followed by the addition of 0.5ml of DTNB. This mixture was then transferred to a spectrophotometer cell. In the reference cell phosphate buffer was taken.

The DTNB blank consisting of 2.5ml of buffer,0.5ml DTNB measured against a reference cell containing 3ml of buffer.

All measurements were taken at 412nm after 5 minutes. Absorbance was recorded on a UV-visible spectrophotometer of Model-1601(-Shimadzu). The glutathione concentrations were calculated relative to the standard curve of the known concentrations of GSH.

Standard curve

Standard curve for GSH was obtained using 200-2000µM GSH by following the standard Ellman's method prescribed above and as shown in the fig. 1.

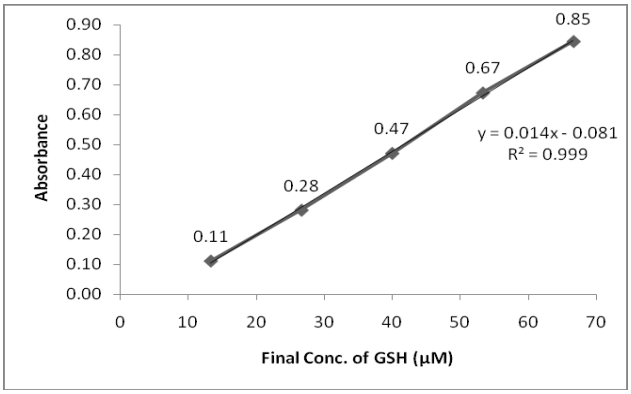

Fig. 1: Standard curve for GSH

RESULTS

Effect of different concentrations of ammonium vanadate on GSH level in plasma

Upon addition of different concentrations (100-1000µM) of Ammonium Vanadate, a persistent decrease in plasma-GSH level was observed. The results for concentration-

dependent decrease in plasma-GSH level by Ammonium Vanadate are shown in fig. 2.

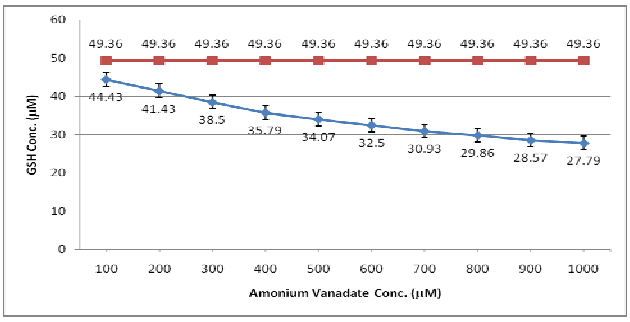

Fig. 2: Effect of NH_4VO_3 concentration on extra cellular plasma GSH content.
■Control: Plasma GSH, ♦ NH_4VO_3 (100-1000µM)
Results are the mean ± SE of 3 experiments of plasma GSH.

Time-dependent effect of different concentrations of ammonium vanadate on GSH level in plasma

After incubation with two different concentrations (100, 1000 µM) of Ammonium Vanadate, a significant change in plasma-GSH level was observed at different time-intervals (0-90 minutes). The results were compared with control plasma, as shown in fig. 3.

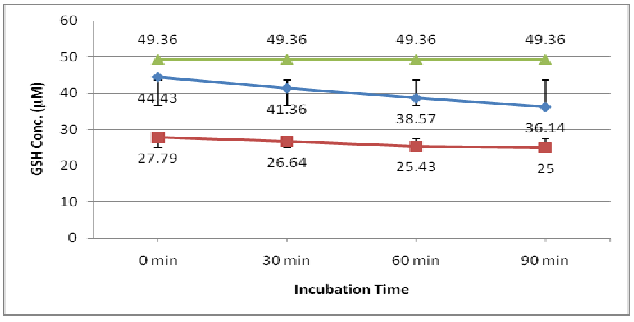

Fig. 3: Effect of NH_4VO_3 concentration on extracellular plasma GSH content with time incubation period 0-90 minutes.
▲ Control: Plasma GSH, ■ $NH_4VO_3$1(100µM)
♦ NH_4VO_3 (1000µM)
Results are the mean ± SE of 3 experiments of plasma GSH.

Ammonium vanadate effect on GSH of intracellular cytosolic fraction of blood

Upon addition of different concentrations (100-1000µM) of NH_4VO_3 to venous blood, measurement of intracellular Cytosolic-GSH content showed gradual decrease, as shown in fig. 4. Intracellular Cytosolic-GSH content was also measured at different incubation time-period (0-90 minutes) after the addition of two different concentrations (100, 1000µM) of NH_4VO_3 to venous blood showing the time-dependent decrease in GSH content as shown in fig. 5.

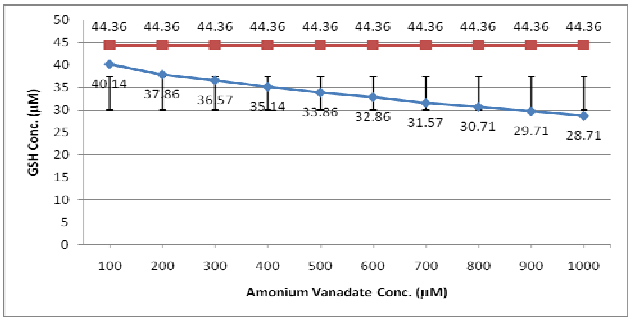

Fig. 4: Effect of NH_4VO_3 concentration on intracellular CF GSH content
■Control: Cytosolic Fraction GSH, ♦ NH_4VO_3 (100-1000 µM)
Results are the mean ± SE of 3 experiments of plasma GSH

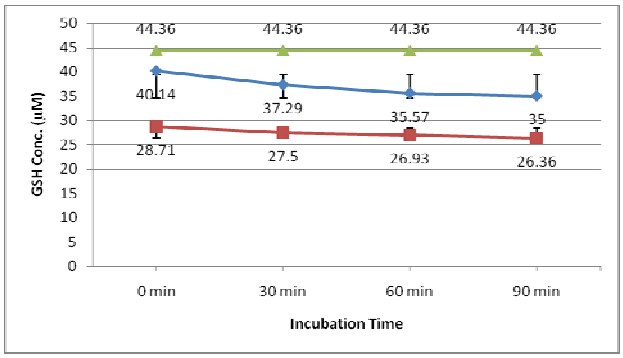

Fig. 5: Effect of NH4VO3 concentration on the intracellular CF GSH content with time incubation period 0-90 minutes
♦ Cytosolic Fraction GSH, ■ NH_4VO_3 (100µM)
▲ NH_4VO_3 (1000µM)
Results are the mean ± SE of 3 experiments of plasma GSH

DISCUSSION

Many metals exhibit a strong affinity for reduced sulfhydryl groups (-SH), forming metal-GSH complexes of high thermodynamic stability (Richardson and Murphy, 1975; Refsvik, 1978; Magos *et al.*, 1978). High affinity of heavy metals for sulfhydryl groups can result in the formation of covalent attachments mainly between heavy metal and sulphydryl groups (Meister, 1988). One major mechanism for metals toxicity appears to be direct and indirect damage to mitochondria via depletion of glutathione, an endogenous thiol-containing (SH-) antioxidant, which results in excessive free radical generation and mitochondrial damage (Sanfeliu *et al.*, 2001). The objective of this studywas to investigate the possible interaction of NH_4VO_3 with the extracellular and intracellular GSH status upon the addition of NH_4VO_3 to the venous blood of healthy human volunteers. Moreover, the effect of heavy metals on the chemical modulation and metabolism of biologically active low molecular weight molecules such as Glutathione (GSH), in biological fluids is an important and active area of

research. The importance of interaction of heavy metals including NH_4VO_3 with GSH in biological fluids as a biomarker of toxicity and detoxification is receiving increasing clinical interest.

Blood components are rich in GSH content. The determination of GSH content in biological fluids before or after incubation with heavy metals have been of value in further understanding of the mechanism of action of heavy metal-induced toxicity.

Results derived from chemical modulation as well as alteration in conjugation and metabolism of GSH in plasma and Cytosolic fraction caused by heavy metals like Vanadium can be applied for human safety evaluations. Results of this study reveal that Treatment of venous blood with NH_4VO_3 and measurement of GSH content in extra and intracellular compartment can indicate the protective role of GSH against V^{+5}. This observation is consistent with previous studies which shows that observations that V^{+5} forms complex with GSH (Sabbioni *et al*., 1992). Our findings offer further evidence that GSH plays a role of protection against V^{+5}. GSH plays a key role in bio-reducing the Vanadium (V^{+5}) to less toxic vanadium (V^{+4}), thereby preventing the toxic effects like neoplastic action of vanadium (V^{+5}). The carcinogenic potential of Vanadium (V^{+5}) strictly depends on its intracellular persistence, where the lack of GSH-mediated reduction provides the opportunity to vanadium (V^{+5}) to remain in the oxidized form (Sabbioni *et al*., 1993). Reduced glutathione (GSH) the major non protein thiol present in animal cells is an extremely important biological reducing agent, involved in the detoxification processes of exogenous material (Robenstein *et al*., 1989) and apparently plays a central role in vanadium metabolism. Besides its reducing potential GSH can act as a ligand for the stabilization of VO^{+2} oxidation (Baran, 2000 and Macara *et al*., 1980).

Our findings show that GSH content were decreased by addition of NH_4VO_3 to venous blood, suggesting the Vanadium (V^{+5})-induced toxicity in blood components. These results are consistent with a role of GSH as a protective mechanism. The mechanism, by which, V^{+5}induces toxicity in our research work is not yet known. From our findings it appears that the oxidative stress or toxic effect of V^{+5} is more exerted at extracellular than intracellular level. This evidence further suggests that extracellular compartment is more oxidized than intracellular compartment. Presumably, GSH provides protection by making a complex with V^{+5}, thus decreasing the availability of $V^{+5}$for toxic effect. This suggests that GSH provides a first line of defense against V^{+5}. The protective role of GSH and the known affinity of GSH for other metal ions may have therapeutic applications. Some of the systems mentioned in this account are evidently relevant to the toxicity and detoxification of vanadium. Some of the metabolic processes (glutathione, ascorbate or cysteine mediated reduction of vanadates (V); complexation of VO^{+2} by different bimolecules; or accumulation of vanadium in hard tissues) must play an important role in biological vanadium detoxification (Baran *et al*., 1998).

The addition of NH_4VO_3 to healthy human venous blood, reduction in GSH content and proposed V-SG complexes and/ or conversion of GSH to GSSG and also a proposed change of V^{+5} to V^{+4}, V^{+2} may account for better than towards the protective role of GSH as well as the pharmacological and toxicological action of vanadium compounds.

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